

## Spermatozeugmata of *Abyssochrysos* : ultrastructure, development and relevance to the systematic position of the Abyssochrysidæ (Prosobranchia, Caenogastropoda)

by John HEALY

**Abstract.** — Light microscopic and electron microscopic examination of testis tissue from the deep-sea gastropod *Abyssochrysos* cf. *eburneum* Locard, reveals the presence of spermatozeugmata. Each spermatozeugma consists of a round, vesicle-filled paraspermatozoon ('nurse cell' type) and numerous euspermatozoa attached via the tips of their acrosomes to the periphery of the paraspermatozoon. Euspermatozoan attachment is restricted to a specific, crescent-shaped area of the paraspermatozoon. Paraspermatozoa lack a nucleus (or nuclear remnant) and mitochondria though both organelles are present in the spermatocytes from which paraspermatozoa develop. Euspermatozoa exhibit the following features : (1) conical acrosome (acrosomal vesicle 1.3-1.7  $\mu\text{m}$  long with apical bleb ; axial rod and basal plate present); (2) rod-shaped nucleus (8-9  $\mu\text{m}$  long, basally invaginated); (3) midpiece (80  $\mu\text{m}$  long, axoneme surrounded by five to seven helically-coiled midpiece elements); (4) glycogen piece (25  $\mu\text{m}$  long, axoneme surrounded by nine wedge-shaped tracts of granules); and (5) annulus (dense ring complex) at junction of midpiece and glycogen piece. All features of euspermiogenesis in *Abyssochrysos* (eg the patterns of acrosomal, nuclear and midpiece development) have previously been reported in a number of other caenogastropod taxa. After comparison with the spermatozoa in other gastropods, it is concluded that the Abyssochrysidæ are closely allied to the Littorinoidea (which produce very similar spermatozeugmata) and its sister group the Truncatelloidea (formerly the Rissooidea). If abyssochrysidæ are truly representative of the Loxonematoidea, that superfamily would occupy a basal position in the evolutionary history of the Caenogastropoda.

**Résumé.** — L'examen structural et ultrastructural du testicule du gastéropode bathyal, *Abyssochrysos* cf. *eburneum* Locard, révèle la présence de spermatozeugma. Chaque spermatozeugma est une vésicule ronde remplie de spermatozoïdes atypiques (de type « cellules nourricières ») portant de nombreux spermatozoïdes typiques accrochés par la pointe de leur acrosome. La surface des spermatozoïdes atypiques présente une spécialisation membranaire en forme de croissant où sont fixés les spermatozoïdes typiques. Les spermatozoïdes atypiques sont dépourvus de noyaux (ou de résidus nucléaires) et de mitochondries bien que les deux types d'organites existent dans les spermatocytes qui leur donnent naissance. Les spermatozoïdes typiques sont caractérisés par : (1) un acrosome conique (vésicule acrosomiale de 1,3-1,7  $\mu\text{m}$  de long avec bulle apicale, baguette axiale et plaque basale); (2) un noyau en forme de bâtonnet (8-9  $\mu\text{m}$  de long, invaginé à sa base); (3) une pièce intermédiaire (80  $\mu\text{m}$  de long, avec axonème entouré de cinq à sept éléments périphériques enroulés en hélice); (4) une pièce à glycogène (25  $\mu\text{m}$  de long, axonème entouré de neuf éléments granulaires en forme de coin); et (5) un annulus (anneau complexe et dense) situé à la jonction de la pièce intermédiaire et de la pièce à glycogène. Tous les caractères de la spermatogénèse typique d'*Abyssochrysos* (par exemple, le type d'acrosome, la différenciation du noyau et de la pièce intermédiaire) ont été précédemment reconnus dans un certain nombre de taxons de Caenogastropodes. De la comparaison avec les spermatozoïdes d'autres gastéropodes, on conclut que les Abyssochrysidæ sont étroitement apparentés aux Littorinoidea (qui

produisent des *spermatozeugma* très semblables) et au groupe voisin des Truncatelloidea (anciennement Rissoidea). Si les Abysochrysidae sont vraiment représentatifs des Loxonematoidea, cette superfamille doit se situer aux origines de l'histoire évolutive des Caenogastropoda.

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## INTRODUCTION

In 1979, HOUBRICK described and discussed the anatomy, distribution and possible relationships of the deep-sea family Abysochrysidae (containing a single genus, *Abysochrysos*). He found that *Abysochrysos* differed significantly in soft part and radular morphology from other caenogastropods, but that shells showed remarkable similarity to those of the 'extinct' Loxonematoidea and to a lesser degree, shells of thiarid cerithioideans. HOUBRICK provisionally placed the Abysochrysidae within the Loxonematoidea and suggested that the family was related to the Viviparoidea, Valvatoidea, Truncatelloidea and Cerithioidea. Since the Loxonematoidea are thought to have given rise (either directly or indirectly) to other caenogastropod taxa (KNIGHT *et al.*, 1954; HEALY, 1984; HASZPRUNAR, 1988) and possibly the Pyramidelloidea (KNIGHT *et al.*, 1954, 1960), the anatomy of inferred living members of this group becomes a subject of considerable importance.

Previous studies have demonstrated that sperm ultrastructure reflects the systematic affinities of molluscan taxa (GIUSTI & SELMI, 1982; HEALY, 1982-1989; KOIKE, 1985). In the case of the Gastropoda, such research has been particularly helpful, most notably for identifying the nearest allies of certain 'problem' groups such as the Architectonicidae, Rissoellidae, Omalogyridae and Pyramidellidae (HEALY, 1982*a*, 1987, 1988*a, b*).

Through the courtesy of Dr. Philippe BOUCHET of the Muséum national d'Histoire naturelle (Paris), the author has had opportunity to examine the ultrastructure of mature and developing spermatozoa in *Abysochrysos* collected off the Brazilian coast during May 1987. The results of this work are presented here, and discussed in terms of comparative sperm morphology and possible taxonomic and phylogenetic significance. Most of the classificatory changes proposed by PONDER & WARÉN (1988) have been used in this study, including elevation of the Caenogastropoda to superordinal status (embracing orders Architaenioglossa, Neotaenioglossa and Neogastropoda).

## MATERIALS AND METHODS

This study was carried out using sea water/formalin-fixed specimens of *Abysochrysos* cf. *eburneum* Locard, 1896, on loan to the author from the Muséum d'Histoire naturelle (Laboratoire de Biologie des Invertébrés Marins et de Malacologie). These specimens were collected from a depth of 960 m off Brazil (station CB 95, 19°38' S, 38°43' W) during the cruise MD 55/Bresil of the Research Vessel "Marion-Dufresne" (30 May, 1987).

Small (1-2 mm<sup>3</sup>) pieces of testis tissue were taken from males and washed for 2 hrs in filtered sea water to remove formalin. The tissues were post-fixed for 1 hr at 0-4° C in a 1 % solution of osmium tetroxide (prepared in sea water), then rinsed in filtered sea water for 2 hrs,

dehydrated via a graded ethanol series, and finally embedded in Spurr's epoxy resin. Survey sections and ultrathin sections were cut using an LKB IV Ultratome. Ultrathin sections were collected on uncoated 200 mesh copper grids, stained with 2-6 % aqueous uranyl acetate and Reynold's lead citrate, and examined using either a Philips 300 or a Hitachi 300 transmission electron microscope (operated at 80 kV). An Olympus II A light microscope was used for observations and photography of survey sections (stained with 0.1 % aqueous toluidine blue) and tissue squashes (phase-contrast light microscopy).

## RESULTS

### PARASPERMATOZOA AND SPERMATOZEUGMATA

Paraspermatozoa of *Abyssochrysos* are spherical to ovoid and approximately 17-18  $\mu\text{m}$  in diameter (pl. I A-C). They contain numerous, moderately electron-dense vesicles (average diameter 5-6  $\mu\text{m}$ ) (pl. I A-D), a central deposit of cytoplasm and a few mitochondria, but no nucleus/nuclear remnant, endoplasmic reticulum, centrioles or axonemes. Clumps of euspermatozoa — perhaps as many as 150-250 (a precise number could not be obtained in available material) — are attached to a specific crescentic site on each paraspermatozoon, to form a mature spermatozeugma (pl. I B also VII B). Euspermatozoal attachment involves contact between the apical bleb of the acrosome and the paraspermatozoon plasma membrane (pl. I D-F). Actual physical enclosure of the euspermatozoan acrosome by the paraspermatozoon is minimal: presumably interaction between euspermatozoal and paraspermatozoal glycocalices plays a major role in maintaining the integrity of each spermatozeugma.

### EUSPERMATOZOA

Euspermatozoa of *Abyssochrysos* consist of an acrosome, nucleus, midpiece, glycogen piece and end piece with enveloping plasma membrane (pl. II A-I).

The acrosomal vesicle is conical, and 1.3-1.7  $\mu\text{m}$  long with a deep basal invagination occupied in part by an axial rod (length 1-1.2  $\mu\text{m}$ , maximum diameter 0.3  $\mu\text{m}$ ). Visible within the wall of the vesicle are elongate plates (pl. II A, C) which, in transverse section, are radially arranged (pl. II A). The membrane of the acrosomal vesicle is dilated apically to form an apparently empty bleb (pl. I E, F; II C). It is this region of the acrosome that attaches to the paraspermatozoon periphery in spermatozeugmata. An accessory membrane surrounds the lower half of the acrosomal vesicle (pl. II C). Positioned between the acrosome and nucleus is a centrally-perforate plate, the anterior surface of which is grooved to fit the basal rim of the acrosomal vesicle (pl. I E; II C).

The nucleus is cylindrical, 8-9  $\mu\text{m}$  long, weakly indented anteriorly and invaginated to a depth of 0.7-0.8  $\mu\text{m}$  posteriorly (pl. II C-E). Located within the posterior invagination are the centriolar derivative and initial portion of the axoneme. Many sperm nuclei are distinctly fibrous in appearance (pl. I E; II C). This may indicate the true substructure of the nucleus, though exaggerated by long-term fixation in sea water formalin.

The midpiece is approximately  $80\ \mu\text{m}$  long and composed of five to seven mitochondrial elements helically coiled around a  $9 + 2$  pattern axoneme (pl. II E-J). A single membrane surrounds the axoneme, while a double membrane (unfortunately stripped from many observed euspermatozoa) surrounds the midpiece elements (pl. II E, G, H). Cristae are visible in each of the coiled elements, but are never well developed (pl. II G, H).

A ring complex or annulus occurs at the midpiece-glycogen piece junction (pl. II I). This complex is composed of : (1) a simple ring attached to the plasma membrane ; and (2) a sleeve of dense material which lines the inner mitochondrial membrane (pl. II I). Some sections also suggest that connections may exist between the ring complex and axoneme.

The glycogen piece consists of the axoneme surrounded by : (1) granular deposits (here interpreted as glycogen : this material largely absent in euspermatozoa examined, either due to dissolution during long-term fixation or slight immaturity of sperm examined); (2) nine radiating spokes (probably granule supports) and (3) the plasma membrane. This region measures approximately  $25\ \mu\text{m}$  (pl. II, F, I, J).

#### EUSPERMATOZOON DEVELOPMENT

Euspermatogenesis in *Abysochrysos* proceeds from spermatogonia of the germinal epithelium in each seminiferous tubule. Spermatogonia are oblong cells ( $5\ \mu\text{m} \times 9\ \mu\text{m}$ ) with an oval nucleus (max. diam.  $6\text{-}7\ \mu\text{m}$ ), bunched mitochondria and scattered endoplasmic reticular (ER) cisternae (pl. III A). The nucleoplasm of spermatogonia is coarsely granular in appearance, with two well-defined nucleoli (both showing a dense medulla and less-dense cortex — pl. III A).

Spermatogonia give rise to primary spermatocytes which, like spermatogonia, also show prominent nucleoli (often two visible). Primary spermatocytes however are distinguished by numerous irregular dense patches within the nucleoplasm and well developed perinuclear ER cisternae (pl. III B).

In secondary spermatocytes the oval-shaped nucleus (max. diam.  $8\text{-}9\ \mu\text{m}$ ) exhibits chromosome cores (pl. III C) and usually one nucleolus. Secondary spermatocytes are interconnected by cytoplasmic bridges and possess two centrioles (pl. III D) and a cluster of oblong mitochondria (pl. III C).

Nuclei of newly-formed euspermatids are compact, usually irregular in shape and very electron dense (pl. III E). Early spermatid nuclei gradually return to an oval or spherical shape and the nucleoplasm assumes a mottled appearance with a granular central portion (pl. III E ; IV A, B). Numerous morphological changes occur almost simultaneously within early euspermatids. Firstly the flagellum forms from a centriole positioned close to the plasma membrane (pl. IV A, B). Nuclear condensation commences posteriorly and peripherally and progresses anteriorly and internally, thereby converting the nuclear contents into a sponge-like reticulum (reticular-fibrillar condensation phase) (pl. IV E). The basal invagination of the nucleus first becomes evident as a slight indentation of the nuclear surface (pl. IV A). As the invagination deepens (during the reticular-fibrillar condensation phase) it forms an insertion and attachment point for the centriole-flagellum apparatus (pl. IV E).

Development of the acrosome begins at the onset of nuclear condensation. The Golgi complex produces two acrosomal components — the basal granule and the acrosomal vesicle.

Plate V A shows the earliest observed stage of acrosome formation : the acrosomal vesicle is attached to the basal granule close to the concave face of the Golgi complex. A newly formed secretory vesicle proceeds from the Golgi cisternae towards the acrosomal vesicle, to which it presumably fuses. Development of the acrosome occurs at some distance from its eventual attachment site at the nuclear apex, but always in close proximity to the Golgi complex (pl. V B, C). The vesicle of the developing acrosome invaginates basally thereby transforming the dense component of the vesicle into a cylindrical structure (pl. V B, C).

As the nucleus passes from the reticular-fibrillar to longitudinal-fibrillar condensation phase, the acrosome attaches to the nuclear apex (pl. V D) and the mitochondria, which formerly were round or pyriform (pl. IV D, E), now begin to elongate posteriorly (pl. V D). In advanced euspermatids the longitudinally-orientated fibres of the nucleus fuse laterally to produce lamellae (longitudinal-lamellar condensation phase (pl. V E) which gradually compact. The midpiece of these advanced cells has almost reached its definitive shape as the mitochondria form an extremely elongate, periaxonemal sheath associated with cytoplasmic microtubules (pl. VI A, B). It is during this last stage of midpiece development that the mitochondria become helically twisted around the axoneme thereby forming the 5-7 midpiece elements of the mature euspermatozoon. As already described, cristae are retained in the mature midpiece (see pl. II E, G, H), though they are not as readily detectable as those from the preceding euspermatids.

The centrally-perforate basal plate of the mature acrosome (pl. I E; II C) is derived from the basal granule of the developing acrosome (pl. V B, D), while the axial rod (a form of subacrosomal deposit) is probably formed from material occupying the vesicle invagination of developing acrosomes or from the basal granule (pl. V B, C).

#### PARASPERMATOZOON DEVELOPMENT

Paraspermatozoa develop from primary spermatocytes which, at least morphologically, are indistinguishable from those producing secondary spermatocytes of the euspermatozoal line. The first signs of differentiation in the paraspermatozoon line are lobulation of the nucleus and a marked increase in cytoplasmic volume (pl. VI D). As the nucleus becomes irregular in shape, it begins to slowly fragment through the pinching-off of lobes (pl. VI C). Plate VI C shows a developing paraspermatozoon with its voluminous cytoplasm and a largely-intact nucleus. Although at least three Golgi complexes are present at this growth stage, production of the numerous developing secretory vesicles (diameted 0.5-1.5  $\mu\text{m}$ ) appears to take place exclusively within the dilated cisternae of the endoplasmic reticulum (pl. VI D). Mitochondria are small (0.3-0.5  $\mu\text{m}$  in length), oblong and scattered among the secretory vesicles (pl. VI D). Presumably by a process of amalgamation, (? facilitated by Golgi activity), the secretory vesicles form the large vesicles observed in mature paraspermatozoa (see pl. I A-D; VII B, C). In immature paraspermatozoa these vesicles frequently show a reticulate pattern of dense and less-dense areas (pl. VII A), but this substructure is not retained. Formation of spermatozeugmata only occurs after paraspermatozoa and euspermatozoa are fully developed.

## DISCUSSION

### PARASPERMATOZOA/SPERMATOZEUGMATA

Mature paraspermatozoa of *Abyssochrysos* strongly resemble those of littorinid genera such as *Littorina* (s.s.), *Littoraria* (subgenus *Littorinopsis*), *Nodilittorina* and *Melaraphe* (REINKE, 1912; BUCKLAND-NICKS & CHIA, 1977; REID, 1986; HEALY unpublished data). Observed similarities between paraspermatozoa of *Abyssochrysos* and littorinids include: (1) spheroidal form; (2) internal structure (large secretory vesicles, absence of nuclear material and axonemes); (3) absence of emergent tail(s) and (4) formation of spermatozeugmata by attachment of euspermatozoa (via tips of acrosomes) to a crescentic site on the paraspermatozoon. THIRIOT-QUIEVREUX & MARTOJA (1979), using light microscopy, reported spermatozeugmata similar to littorinids and *Abyssochrysos* in *Carinaria lamarki* (Pterotracheoidea — ‘Heteropoda’) but only euspermatozoa were found in another pterotracheoid *Atlanta gaudichaudi* by JAMIESON & NEWMAN (1989) using electron microscopy. Further electron microscopical work will be needed to determine the incidence of spermatozeugmata formation within the Pterotracheoidea, and the level of similarity to spermatozeugmata of littorinids and *Abyssochrysos*.

Spermatozeugmata occur in other caenogastropods, notably the Janthinoidea (BULNHEIM, 1962; NISHIWAKI & TOCHIMOTO, 1967; MELONE *et al.*, 1978), Triphoroidea (Cerithiopsidae — FRETTER & GRAHAM, 1962; HEALY, 1989; Triphoridae — HEALY, 1989) and *Fusitritou* (Cymatiidae — BUCKLAND-NICKS *et al.*, 1982). In these taxa however, euspermatozoa are attached to mobile, multiaxonemal paraspermatozoa. Spermatozeugmata presumably assist in prevention of premature dispersal of cuspermatozoa before and/or after copulation (eg littorinids — BUCKLAND-NICKS & CHIA, 1977), or in the case of aphyllid, though still internally-fertilizing, taxa such as the Triphoroidea and Janthinoidea, these cellular associations may perform additionally a spermatophoral function (eg GRAHAM, 1954). Unlike littorinids or *Carinaria*, *Abyssochrysos* lacks a penis (see PONDER & WARÉN, 1988) though HOUBRICK (1979) mentions an “accessory copulatory process” in his description of *A. melanoides* anatomy (based on rehydrated specimens).

The secretory vesicles of paraspermatozoa of *Abyssochrysos*, littorinids and *Carinaria* and paraspermatozoa of other caenogastropods probably play an important role in the nutrition of euspermatozoa within the female reproductive tract (eg REINKE, 1912; BUCKLAND-NICKS & CHIA, 1977) or contribute to nutrition of the female or egg production (re-use of vesicles contents).

### EUSPERMATOZOA, EUSPERMATOGENESIS

The features of mature and developing euspermatozoa of *Abyssochrysos* differ in no way from those described in many other caenogastropod taxa (eg — some Littorinoidea and Truncatelloidea; Vermetoidea; Stromboidea; Cypraeoidea; Naticoidea; Janthinoidea; Calyp-

tracoeidea; some neogastropods — see KOHNERT & STORCH, 1984*a*; KOIKE, 1985; HEALY, 1988*a*). In the Cyclophoroidea, Viviparoidea and Cerithioidea (excepting *Campanile* — HEALY, 1986*b*), midpiece development culminates in the formation of parallel plate-like cristae within each of the periaxonemal elements (four straight elements in Cerithioidea, four helically-coiled elements in most Viviparoidea, seven or eight straight elements in Cyclophoroidea) (KAYE, 1958; HEALY, 1982*b*; SELMI & GIUSTI, 1980). Although few ultrastructural studies of spermatogenesis in the Caenogastropoda give details of pre-spermiogenic stages, those that do (eg WALKER & MACGREGOR, 1968; BUCKLAND-NICKS & CHIA, 1976; WEST, 1978; GRIFFOND, 1980; CLAVIERA & ETGES, 1988) are essentially in agreement with results presented here.

#### SYSTEMATIC CONSIDERATIONS : ABYSSOCHRYSID RELATIONSHIPS

Prior to HOUBRICK's (1979) study of *Abyssochrysos*, most authors included the Abyssochrysidae within the Cerithioidea (e.g. WENZ, 1938; TAYLOR & SOHL, 1962), though sometimes with reservations (e.g. THIELE, 1931; FRANC, 1968). GOLIKOV & STAROBOGATOV (1975) however, excluded the Abyssochrysidae from the Cerithioidea because of the presence of what they termed a "cephalic copulatory apparatus" (p. 219). They removed the family to their superorder Littorinimorpha, tentatively close to the Rissoidae, Rissoidae and 'Anabathronidae'. Although more recent work has shown that the pallial process of *Abyssochrysos* is in fact a tentacle and not a penis (see PONDER & WARÉN, 1988), the presence of closed genital ducts does support removal of the Abyssochrysidae from the Cerithioidea (see HOUBRICK, 1979, for further discussion). HOUBRICK (1979 : 19) concluded that soft-part anatomy and radular morphology of *Abyssochrysos* were "indicative of simple mesogastropods related to the superfamilies Viviparacea, Valvatacea, Rissoacea and Cerithiacea". He clearly demonstrated a close resemblance between abyssochrysid shells and those of the Loxonematoidea (especially Pseudozygopleuridae) — a group believed to have been extinct since the upper Jurassic — and provisionally assigned the Abyssochrysidae to that superfamily.

Sperm morphology and spermiogenesis contributes substantially to our knowledge of the relationships of the Abyssochrysidae to other prosobranch gastropods. The features of euspermiogenesis in *Abyssochrysos* are as observed in numerous other caenogastropods (KOHNERT & STORCH, 1984*b*; KOIKE, 1985; HEALY, unpublished observations). Mature euspermatozoa closely resemble those of some Littorinoidea (*Bembicium*, *Laevilitorina*, *Lacuna*), the Vermetoidea, some Truncatelloidea (eg Vitrinellidae), Cypraeoidea, Naticoidea, Stromboidea, Janthinoidea, Triphoroidea, Calyptraeidea and certain neogastropod taxa (Volutidae, Turbinellidae) (KOHNERT & STORCH, 1984*a*; KOIKE, 1985; HEALY, 1986*c*, 1988*a*, *c*, 1989, unpublished observations). Sperm morphology does not support HOUBRICK's view that the Viviparoidea, Cerithioidea and Valvatoidea could be close relatives of the Abyssochrysidae. The Viviparoidea, true Cerithioidea and Cyclophoroidea show euspermatozoan and paraspermatozoan features which separate these three taxa, as a group, from *Abyssochrysos* and other caenogastropods (HEALY, 1982*b*, 1983, 1986*a*, *b*, 1988*a*; HEALY & JAMESION, 1981). The Valvatoidea exhibit a number of unusual sperm characters, some possibly unique to that superfamily and some suggestive of close links with the Heterobranchia (= Euthyneura) rather than the Archaeogastropoda or Caenogastropoda (HEALY, unpublished observations)

supporting the anatomical conclusions of SALVINI-PLAWEN & HASZPRUNAR (1987), RATH (1988) and HASZPRUNAR (1988). Of particular interest is the production of littorinid-type paraspermatozoa and spermatozeugmata in *Abyssochrysos* (for comparison see REINKE, 1912; BUCKLAND-NICKS & CHIA, 1977; REID, 1986). HOUBRICK (1979), though aware of TOMLIN'S (1931) suggestion that abyssochrysid may be allied to the Littorinidae and Thiaridae<sup>1</sup>, did not discuss the possibility of an abyssochrysid-littorinid connection. GOLIKOV & STAROBOGATOV (1975) were uncertain as to the correct systematic position of the Abyssochrysidae, but placed that family, the Truncatelloidea (as Rissooidea — split by them into several superfamilies) and the Littorinoidea, into a suborder Littorinina of their superorder Littorinimorpha (a higher category embracing several neotaenioglossan superfamilies including Vermetoidea, Stromboidea, Pterotrachaeoidea, Dolioidea, Calyptraeidea, Cypraeoidea, Naticoidea). Anatomical studies suggest that Littorinoidea, Cingulopsoidea and possibly Truncatelloidea are primitive neotaenioglossans and closely related to each other (FRETTER & GRAHAM, 1962; PONDER, 1983, 1988).

If abyssochrysid are accepted as living members of the Loxonematoidea, as they have been in some recent classifications (HOUBRICK, 1979; BOSS, 1982; provisionally by PONDER & WARÉN, 1988), then spermatozeugmata and euspermatozoan morphology would support a basal position for this superfamily in caenogastropod phylogeny — in close association with the Littorinoidea, Cingulopsoidea and Truncatelloidea. This conclusion is in accord with the view (HEALY, 1984, 1988c) that the Loxonematoidea, through adaptive radiation in the late Paleozoic and/or early Mesozoic, may have given rise to numerous other caenogastropod taxa.

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1. It should be emphasized that thiarid euspermatozoa and paraspermatozoa are of the cerithioid type (see KOHNERT & STORCH, 1984a, b). Aside from some conchological similarities between thiarids and abyssochrysid, there are no grounds for associating these two taxa.



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PLATE I

*Abyssochrysos* : spermatozeugmata.

- A — Paraspermatozoon showing constituent secretory vesicles and central deposit of cytoplasm. Euspermatozoa also visible in figure ( $\times 5,600$ ).
- B — Spermatozeugmata in testis (light microscopy, toluidine-blue stained resin sections). Each paraspermatozoon bears numerous euspermatozoa (attached via their darkly staining heads) to form a mature spermatozeugma ( $\times 500$ ).
- C — Phase-contrast light micrograph of paraspermatozoon (without attached euspermatozoa) showing secretory vesicles ( $\times 1,250$ ).
- D — Periphery of a spermatozeugma. Euspermatozoa cut in transverse section (arrows) are attached via their acrosomes to the paraspermatozoon ( $\times 31,400$ ).
- E, F — Acrosomal region of attached euspermatozoa. Note apical bleb and centrally perforate basal plate ( $\times 43,200$ ;  $47,800$ ).

*Abbreviations used in figures*

a, acrosomal complex (mature or developing); ab, apical bleb of acrosomal vesicle; am, accessory membrane (of acrosomal complex); an, annulus; ar, axial rod (of acrosomal complex); av, acrosomal vesicle; ax, axoneme; bp, basal plate (of acrosomal complex); c, centriole; eu, euspermatozoa; f, flagellum of euspermatid; G, Golgi complex; gp, glycogen piece; m, mitochondria; M, midpiece; mpe, midpiece element; mt, microtubules; n, nucleus; nc, nucleolus; nf, nuclear fragment (in developing paraspermatozoon); spc, secondary spermatocytes; sz, spermatozeugmata (paraspermatozoon with attached euspermatozoa); v, secretory vesicles of paraspermatozoon.

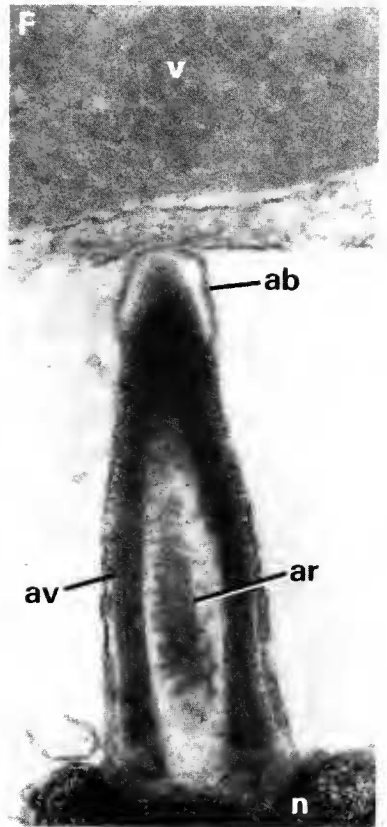
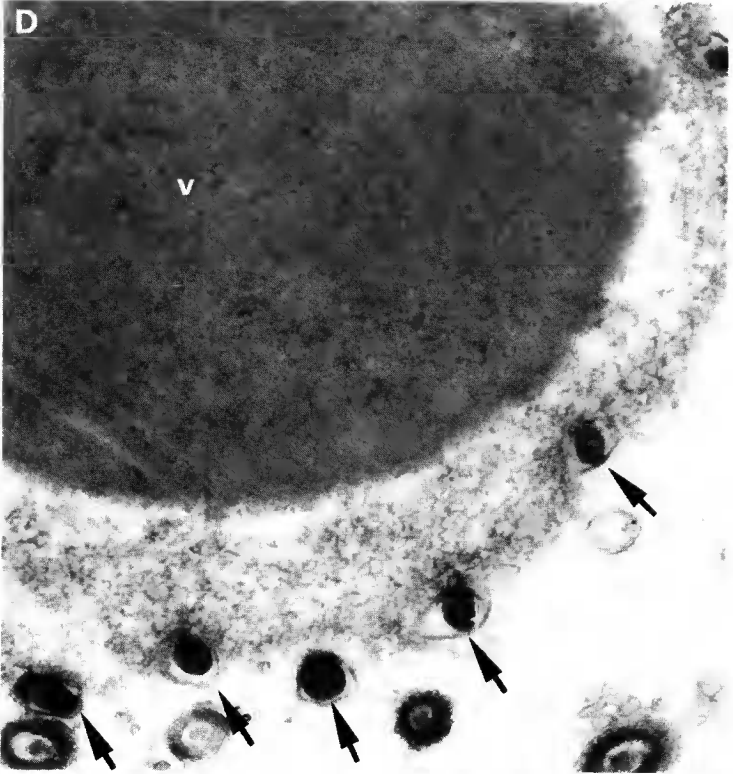
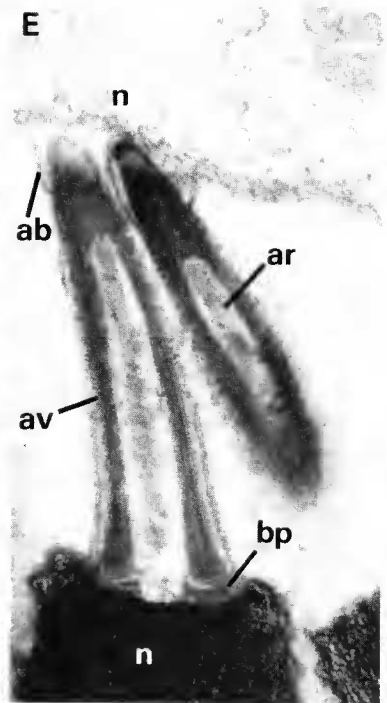
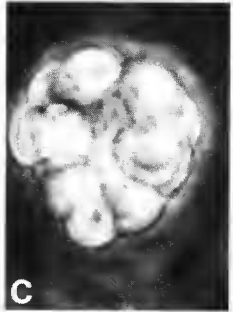
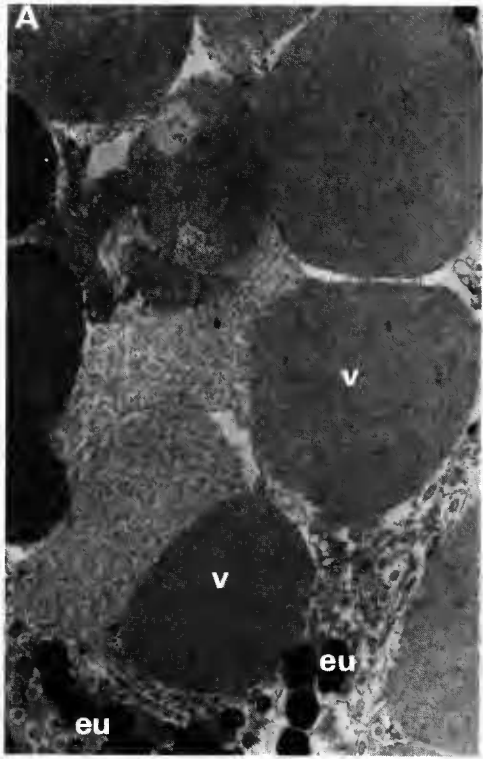


PLATE I

PLATE II

*Abyssochrysos* : cuspermatozoa.

- A, B — Transverse sections (T.S.) through middle (A) and basal (B) portions of the acrosome ( $\times 74,700$ ).
- C — Longitudinal section (L.S.) of acrosome and apex of nucleus. Basal plate has a central perforation ( $\times 40,000$ ).
- D — L.S. cuspermatozoan heads (= acrosome + nucleus) ( $\times 12,000$ ).
- E — L.S. nucleus-midpiece junction. Shallow basal invagination of nucleus contains the centriolar derivative ( $\times 45,000$ ).
- F — Phase-contrast light micrograph showing cuspermatozoon. Arrow indicates junction of midpiece and glycogen piece ( $\times 1,000$ ).
- G — T.S. midpieces. 5-7 helically coiled mitochondrial elements surround the axoneme ( $\times 40,000$ ).
- H — Oblique L.S. showing helical midpiece elements ( $\times 44,000$ ).
- I — Midpiece-glycogen piece junction with annulus ( $\times 50,000$ ).
- J — T.S. glycogen piece. Note spokes for granule support; oblique sections of midpiece also visible ( $\times 47,500$ ).

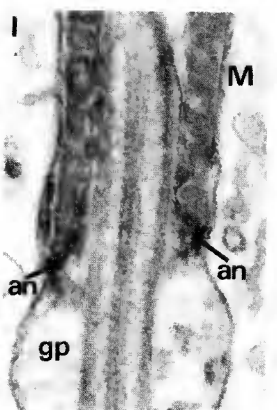
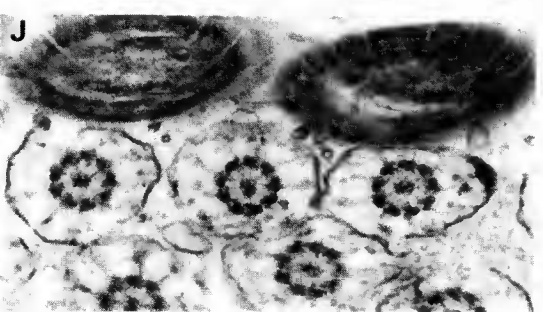
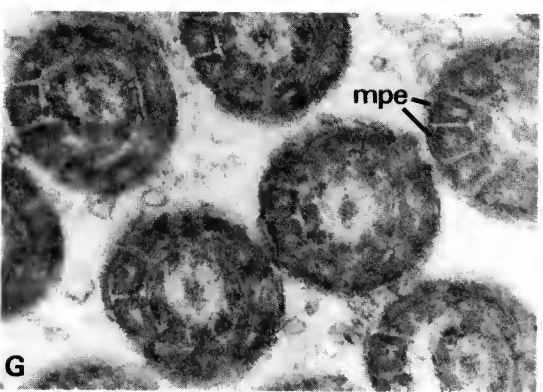
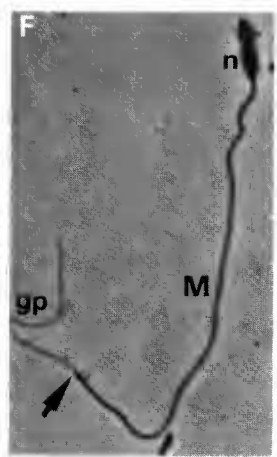
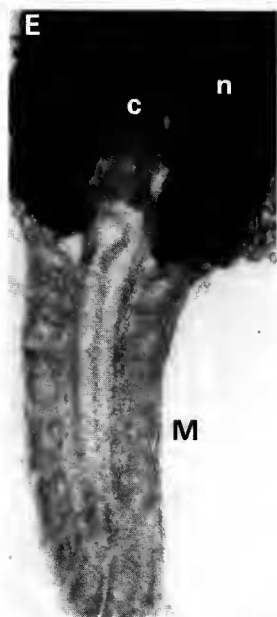
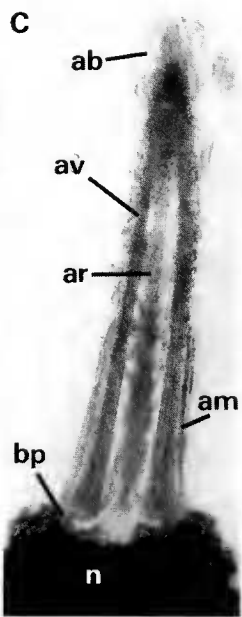
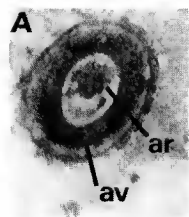


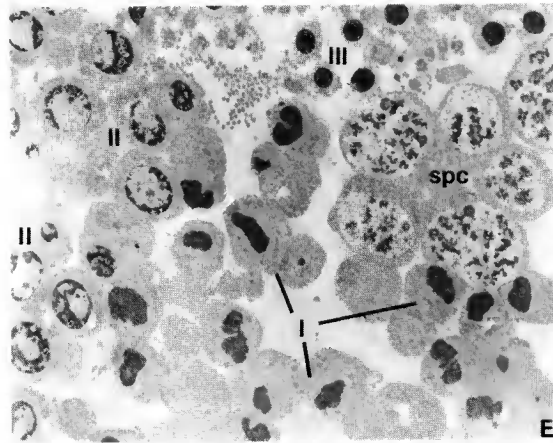
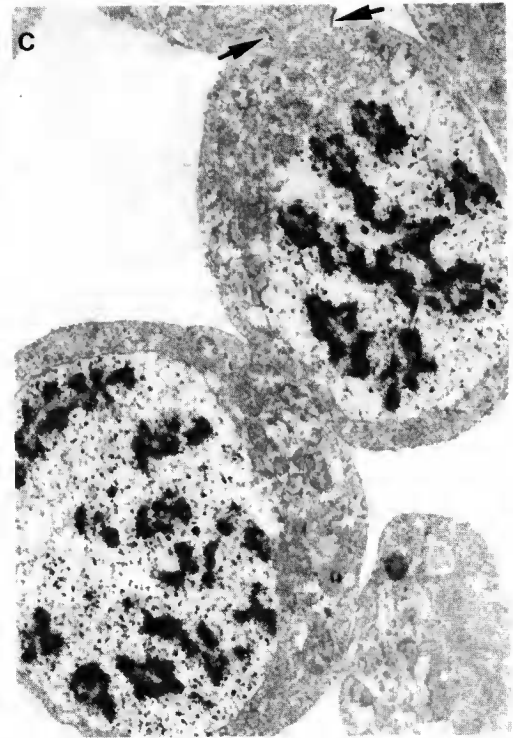
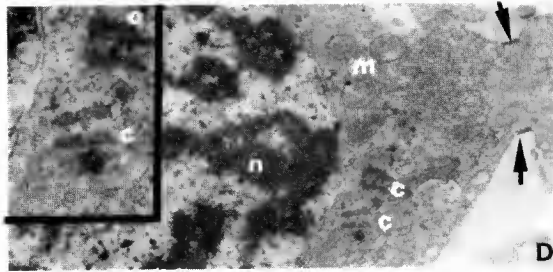
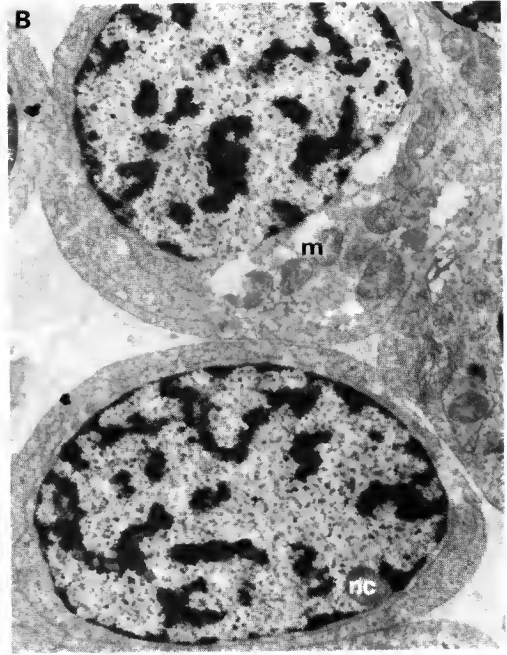
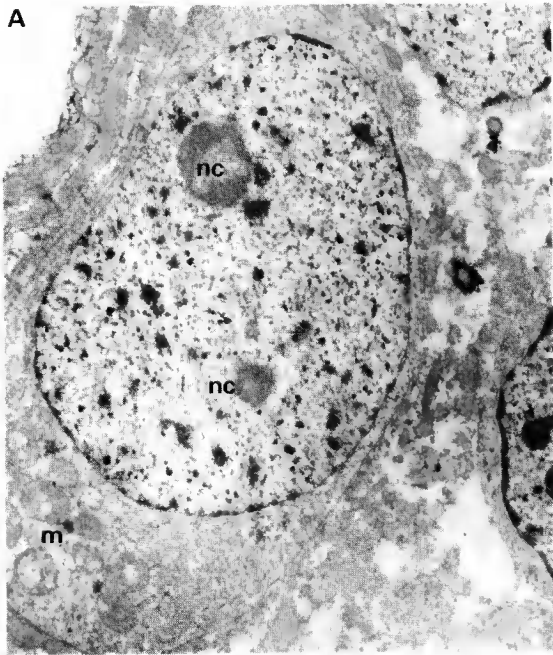
PLATE II

PLATE III

*Abyssochrysos* : euspermatozoon development.

- A — Spermatogonium of the germinal epithelium. Note granular nucleoplasm and presence of two structured nucleoli ( $\times 10,000$ ).
- B — Primary spermatocytes. Nucleoplasm shows dense patches ( $\times 9,000$ ).
- C — Secondary spermatocytes. Note cytoplasmic bridge (arrows). Chromosome cores clearly visible ( $\times 10,150$ ).
- D — Secondary spermatocyte with cytoplasmic bridge (arrows) and paired centrioles ( $\times 14,000$ ). Inset : detail of centrioles ( $\times 28,000$ ).
- E — Survey section of testis showing secondary spermatocytes, newly formed euspermatids (I), euspermatids at onset of nuclear condensation (II), and advanced euspermatids (III) ( $\times 2,100$ ).





*PLATE III*

PLATE IV

*Abyssochrysos* : euspermatozoon development.

- A — Early spermatid at onset of nuclear condensation. Note flagellum developing from centriole at cell periphery and early stage in formation of nuclear invagination (arrow) ( $\times 21,300$ ). Inset : detail of developing nuclear invagination ( $\times 38,300$ ).
- B — Early spermatid showing flagellum and centriole at cell periphery and (arrow) portion of cytoplasmic bridge ( $\times 18,600$ ).
- C — Cytoplasmic bridge (arrows) between euspermatids ( $\times 16,000$ ).
- D — T.S. through developing midpiece of euspermatid (same stage as pl. IV E). Seven mitochondria elongate posteriorly during this process ( $\times 21,600$ ).
- E — L.S. euspermatid showing development of nuclear invagination (now enveloping centriolar derivative), reticular-fibrillar phase of nuclear condensation, and position of mitochondria (see pl. IV D) ( $\times 27,600$ ).

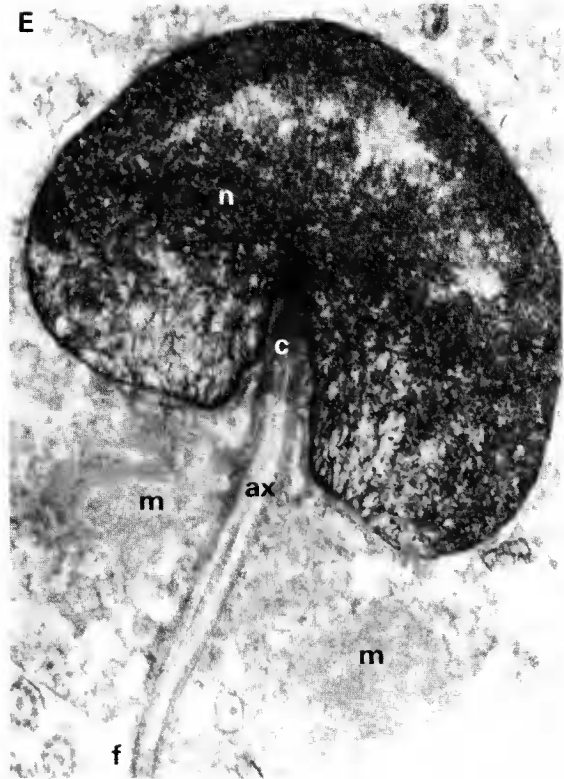
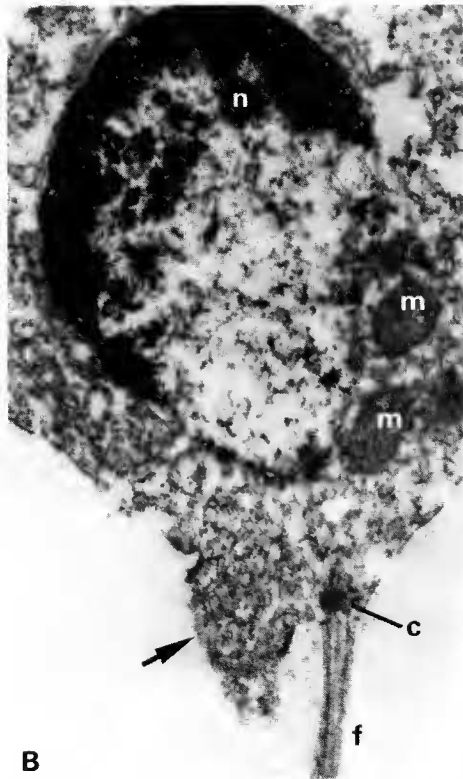
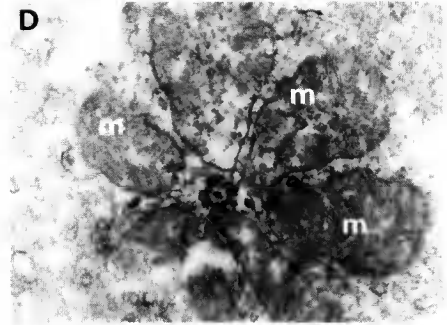
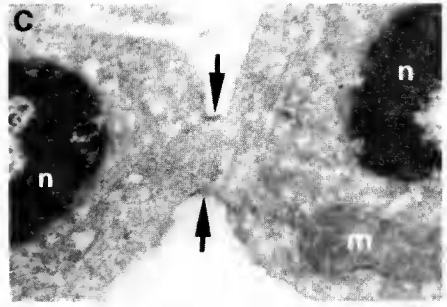
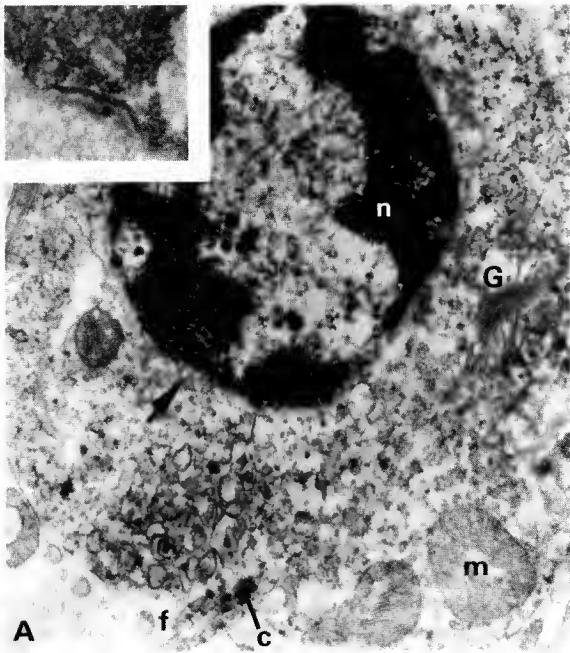


PLATE IV

PLATE V

*Abyssochrysos* : euspermatozoon development.

- A — Formation of the acrosome by the Golgi complex. At this stage the acrosome is composed of a basal granule and developing acrosomal vesicle. Figure also shows a membrane-bound product (arrow) from the Golgi complex presumably about to fuse with the acrosomal vesicle ( $\times 51,500$ ).
- B — More advanced acrosome. Base of the vesicle has invaginated further ( $\times 37,200$ ).
- C — Same stage as pl. V B, showing relative positions of condensing nucleus (see also pl.IV E), developing midpiece and Golgi-acrosome association ( $\times 20,000$ ).
- D — L.S. euspermatid at longitudinal-fibrillar phase of nuclear condensation. Mitochondria have commenced elongation, while anteriorly the acrosome tilts into position at the nuclear apex ( $\times 24,400$ ).
- E — L.S. Nucleus-midpiece junction of advanced stage euspermatid. The nucleus is at the longitudinal-lamellar phase of condensation. Elongate mitochondria now form the midpiece elements ( $\times 24,400$ ). Inset : detail nuclear lamellae ( $\times 54,800$ ).

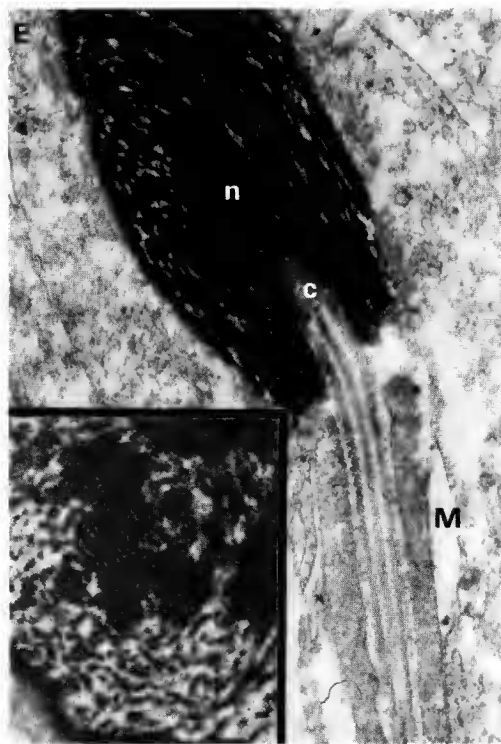
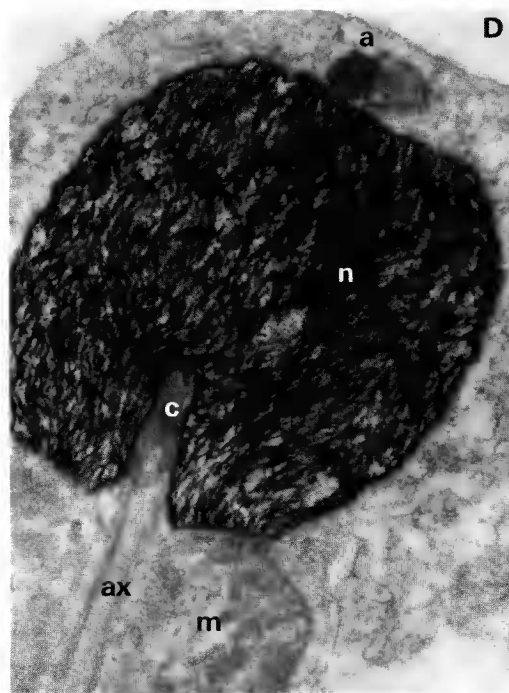
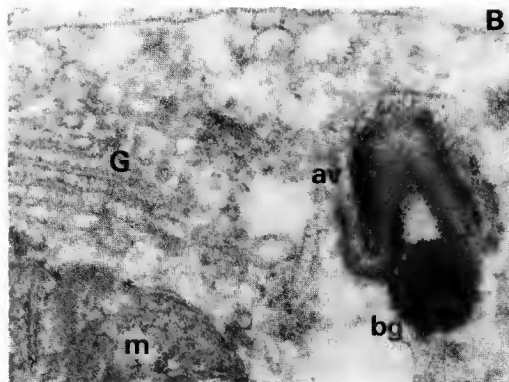
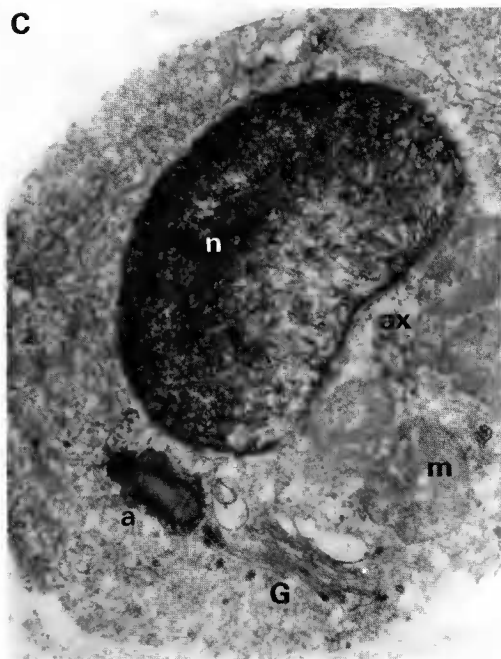
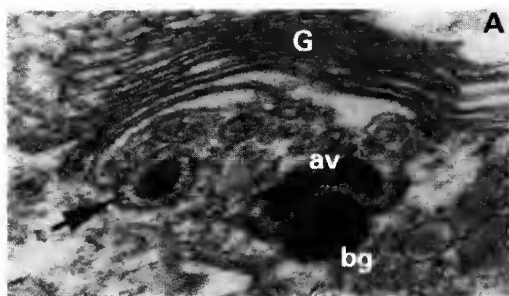


PLATE V

PLATE VI

*Abyssochrysis* : euspermatozoon development (concluded); paraspermatozoon development.

- A — T.S. late euspermatid midpiece showing cristae in periaxonemal element ( $\times 27,800$ ).
- B — T.S. late euspermatid midpiece with surrounding microtubules in cytoplasmic sheath ( $\times 27,000$ ).
- C — Early stage of paraspermatozoon development (from primary spermatocytes). Note nucleolus, nuclear fragments, mitochondria, and lobulate (fragmenting) nucleus ( $\times 14,800$ ).
- D — Developing paraspermatozoon showing three Golgi complexes, mitochondria and numerous developing secretory vesicles (forming within dilated portions of the endoplasmic reticulum) ( $\times 8,700$ ).

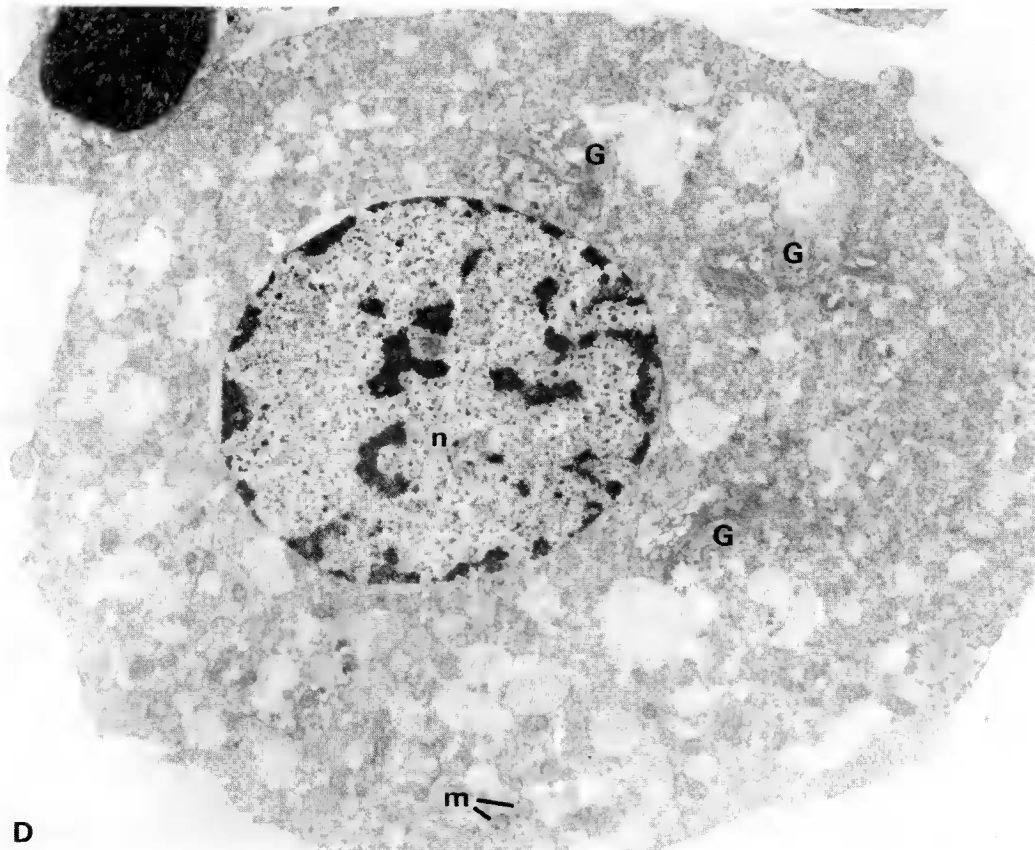
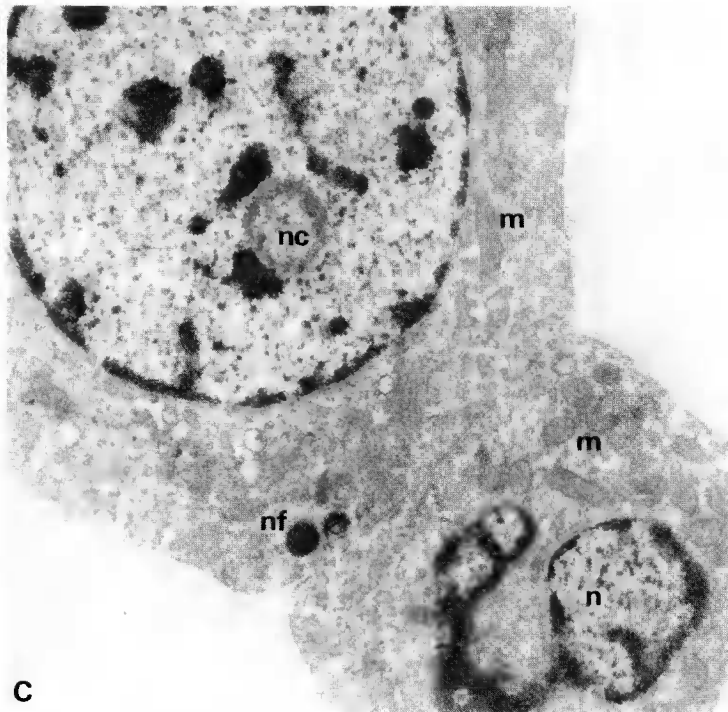
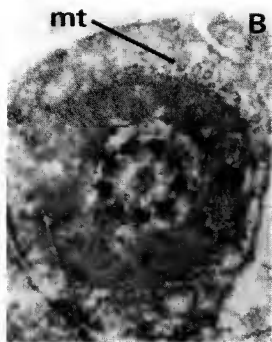


PLATE VII

*Abyssochrysis* : paraspermatozoon development (concluded).

- A — Almost mature paraspermatozoon showing vesicles at various stages of compaction, and scattered, dense nuclear fragments ( $\times 10,500$ ).
- B — Light micrograph showing spermatozeugmata and developing paraspermatozoa and euspermatids within the testis (toluidine-blue stained resin sections) ( $\times 500$ ).
- C — Almost mature paraspermatozoon — note extensive cytoplasmic deposit and differing electron-density of secretory vesicles ( $\times 5,500$ ).



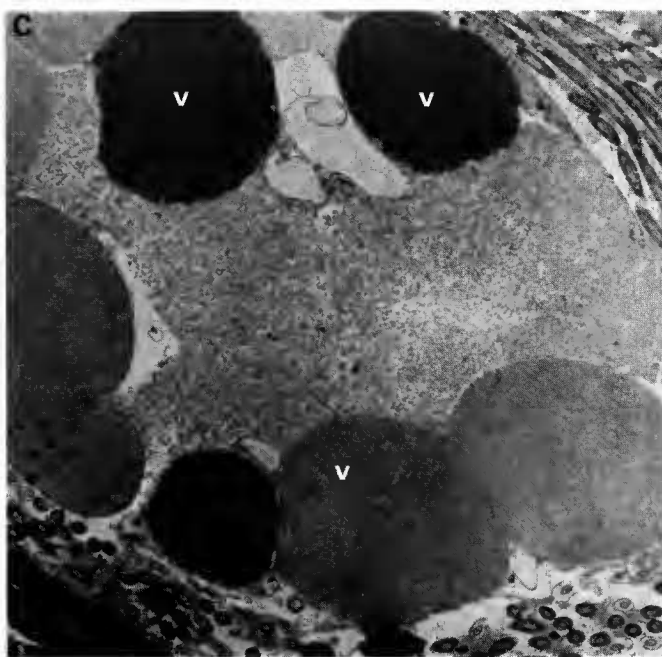
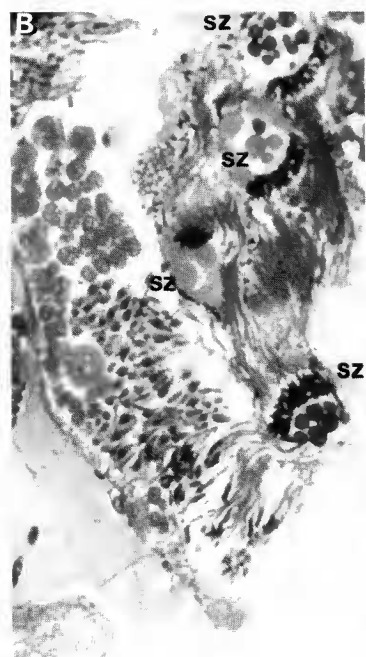
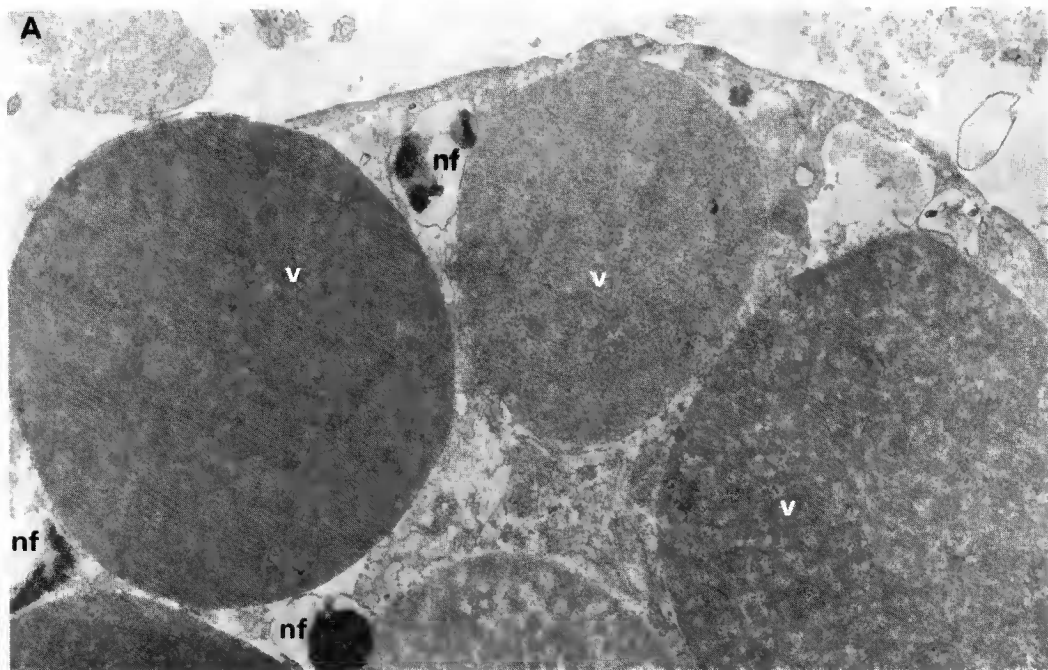


PLATE VII